duced our finding of BAT-26 polymorphisms and the increased frequency of these polymorphisms in African-Americans.[3]

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References

1. Samowitz WS, Slattery ML, Potter JD, Leppert MF: BAT-26 and BAT-40 instability in colorectal adenomas and carcinomas and germline polymorphisms. Am J Pathol 1999, 154:1637–1641
2. Brownstein MJ, Carpten JD, Smith JR: Modulation of non-templated nucleotide addition by TaqDNA polymerase: primer modifications that facilitate genotyping. BioTechniques 1996, 20:1004–1010
3. Pyatt R, Chadwick RB, Johnson CK, Adebamowo C, de la Chapelle A, Prior TW: Polymorphic variation at the BAT-25 and BAT-26 loci in individuals of African origin: implications for microsatellite instability testing. Am J Pathol 1999, 155:349–353

The Role of NAC in Amyloidogenesis in Alzheimer's Disease

To the Editor-in-Chief:

In 1993, Saitoh and colleagues identified the non-Aβ component of Alzheimer's disease (AD), amyloid (NAC), as an important element of amyloid-enriched fractions in AD brains.[1] NAC is a 35-amino acid (aa) fragment derived from its 140-aa precursor, α-synuclein, which is now recognized to play a major role in Lewy body disease (LBD) pathogenesis.[2] Furthermore, immunohistochemical studies with the anti-NAC-X1 antibody revealed that NAC is closely colocalized with Aβ in the AD plaques.[1] Moreover, biochemical studies showed that NAC is extremely hydrophobic and easily forms amyloid-like fibrils under physiological conditions.[3] These results led us to the hypothesis that NAC might play an important role in Aβ aggregation and amyloidogenesis in AD.[1]

In a recent issue of *The American Journal of Pathology*, Culvenor et al[4] reported that NAC may not be associated with Aβ in the plaques. Using the antibody NAC42580 to label cortical and hippocampal sections of AD cases, the authors found no evidence of NAC immunoreactivity in the plaques. Furthermore, although the NAC42580 showed certain immunoreactivity in the urea extracts of the sodium dodecyl sulfate (SDS)-insoluble fractions in diffuse LBD and Parkinson's disease (PD) cases, there was no correlation between NAC and Aβ immunoreactivity in the same AD fractions. Based on this study and on a previous report,[5] the authors concluded that NAC may not be associated with Aβ in the plaques of AD.

Several possibilities could be considered to explain the discrepancy between our results and those reported by Culvenor et al.[4] First, differences in immunoreactivity could be due to differential properties of antibodies used in each study. NAC-X1 and NAC42580 were raised against epitopes derived from different portions of the NAC region: NAC1–9 for NAC-X1[1] and NAC15–31 for NAC42580.[4,5] It is important to note that immunoblotting analysis showed that NAC-X1 preferentially recognizes aggregated forms of the NAC molecule over the monomeric ones.[3] Furthermore, in our experience, NAC-X1 does not immunoreact with soluble α-synuclein.[3] Thus, it can be predicted that our NAC-X1 antibody may be ideal to detect aggregated NAC in the plaques of AD. In contrast, immunoblotting analysis with the NAC42580 showed immunoreactivity with soluble α-synuclein in brain tissues.[4,5] In fact, biochemical analysis of the NAC peptide by El-Agnaf et al[6] showed that the N-terminal region of NAC (NAC1–18) aggregates to form amyloid fibrils, while the C-terminal region (NAC19–35) remains soluble, suggesting that the N-terminal portion is essential for aggregation of NAC peptide.[6] Taken together, these observations might suggest that the NAC1–9 peptide could be naturally aggregated during inoculation in the rabbit, leading to the production of NAC-X1, which preferentially recognizes aggregated forms of NAC, whereas the C-terminal region of NAC may be less effective.

Second, differences in antibody immunoreactivity might depend on the methods used for tissue preparation. In this regard, the NAC-X1 antibody immunolabeled plaques in paraformaldehyde-fixed vibratome sections, but not in formalin-fixed, paraffin-embedded tissue.[1,3,7,8] In fact, both Bayer and Culvenor used archival formalin-fixed and/or paraffin-embedded tissues, rather than vibratome, for their immunohistochemical studies,[4,5] raising the possibility that fixation, solvents, and paraffin might destroy the NAC-X1 epitope. Further supporting the importance of tissue pretreatment and processing to detect the NAC epitope, we have recently shown that in vibratome sections pretreated with formic acid, the NAC-X1 antibody immunostained not only amyloid plaques and amyloid angiopathy, but also astroglial cells and granular neurons in LBD.[7]

Finally, it is possible that the NAC-X1 antibody might be cross-reacting with or recognizing protein quaternary structure. In this regard, immunoblotting analysis confirmed that both monomeric and aggregated forms of Aβ were not immunoreactive with NAC-X1.[1] Therefore, it is unlikely that NAC-X1 cross-reacts with Aβ.

In summary, we would like to argue that it is necessary to continue exploring the hypothesis that NAC plays an important role in plaque formation and AD. The essential difficulty in obtaining conclusive evidence may be due to the lack of information as to the mechanisms by which NAC is generated from its precursor, α-synuclein.[2] In this regard, the results of this study by Culvenor et al[4] are potentially interesting, because their immunoblotting analysis of brain homogenates showed that in addition to

full length α-synuclein at 18kd, NAC42850 recognized faint bands at 12 and 6 kd, supporting the possibility that α-synuclein undergoes degradation. We expect that further characterization of these short fragments might disclose a yet unknown mechanism of α-synuclein proteolysis, leading to a better understanding of the role of α-synuclein and NAC in the pathogenesis of AD.

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References

1. Ueda K, Fukushima H, Masliah E, Xia Y, Iwai A, Otero D, Kondo J, Ihara Y, Saitoh T: Molecular cloning of a novel component of amyloid in Alzheimer's disease. Proc Natl Acad Sci USA 1993, 90:11282–11286
2. Hashimoto M, Masliah E: Alpha-synuclein in Lewy body disease and Alzheimer's disease. Brain Pathol 1999, 9:707–720
3. Iwai A, Yoshimoto M, Masliah E, Saitoh T: Non-Aβ component of Alzheimer's disease amyloid (NAC) is amyloidogenic. Biochemistry 1995, 34:10139–10145
4. Culvenor JG, McLean CA, Cutt S, Campbell BCV, Maher F, Jakala P, HartmannT, Beyreuther K, Masters CL, Li Q-X: Non-Aβ component of Alzheimer's disease amyloid (NAC) revisited: NAC and α-synuclein are not associated with Aβ amyloid. Am J Pathol 1999, 155:1173–1181
5. Bayer TA, Jakala P, Hartmann T, Havas L, McLean C, Culvenor JG, Li Q-X, Masters CL, Falkai P, Beyreuther K: α-synuclein accumulates in Lewy bodies in Parkinson's disease and dementia with Lewy bodies but not in Alzheimer's disease β-amyloid plaque cores. Neurosci Lett 1999, 266:213–216
6. El-Agnaf OM, Bodles Am, Guthrie DJS, Harriott P, Irvine B: The N-terminal region of non-Aβ component of Alzheimer's disease amyloid is responsible for its tendency to assume β-sheet, and aggregates to form fibrils. Eur J Biochem 1998, 258:157–163
7. Takeda A, Hashimoto M, Mallory M, Sundsmo M, Hansen L, Masliah E: C-terminal α-synuclein immunoreactivity in structures other than Lewy bodies in neurodegenerative disorders. Acta Neuropathol 1999, in press
8. Takeda A, Hashimoto M, Mallory M, Sundsmo M, Hansen L, Sisk A, Masliah E: Human NACP/α-synuclein distribution in Lewy body disease. Lab Invest 1998, 78:1169–1177

Author's Reply:

The central questions we have tried to address are whether intact α-synuclein (αSN) or its proteolytic product, the non-Aβ component of Alzheimer's disease (AD) amyloid, NAC, αSN(61–95), ever occurs as an extracellular aggregate, and, if so, whether there is any special relationship with Aβ deposition. Earlier studies by Saitoh, Masliah, and colleagues[1–4] using antibodies to NAC(1–9) indicated that it may be an integral but minor component of Aβ amyloid plaques. Our antibodies to NAC(15–31)[5,6] and NAC(11–23) (unpublished) fail to reveal an association of NAC with the extracellular Aβ amyloid deposits in AD. There is however, intracellular NAC immunoreactivity in abnormal neuritic structures in close association with Aβ amyloid plaques restricted to the CA1 region of the hippocampus in cases of AD combined with cortical Lewy bodies.

The response of Hashimoto and colleagues suggests our data reflect a lack of reactivity with aggregated NAC; however, all our antibodies react with aggregated αSN as found in Lewy bodies. The suggestion that differences may be due to tissue preparation protocols is also unlikely, because our antibodies were reactive with Lewy bodies after formalin fixation, paraffin sectioning, and formic acid treatment. Hashimoto and colleagues themselves report no difference of labeling of vibratome or paraffin sections after formic acid pretreatment of tissue.[4] We therefore suspect that the reactivity of the NAC(1–9) antibody with AD plaques may be due to cross-reactivity with Aβ, since residues 6–8 of NAC share identity with the C-terminal region of Aβ(36–38), as identified earlier by Han et al.[7]

Our Western blot analysis of SDS-insoluble extracts of brain tissue from cases of dementia with Lewy bodies indicates that aggregates of αSN contain full-length as well as truncated species, including a putative NAC fragment of about 6 kd. In pure AD cases, we are unable to demonstrate accumulation of SDS-insoluble αSN or a NAC fragment in the same fractions that contain the SDS-insoluble Aβ peptide.[5] In a further study of four AD cases, SDS-insoluble αSN or the NAC fragment was not detectable (Campbell et al, manuscript submitted). Our combined immunocytochemical and biochemical data therefore indicate that αSN and its proteolytic products accumulate largely in intracellular compartments, independent of extracellular Aβ amyloid deposits.

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References

1. Ueda K, Fukushima H, Masliah E, Xia Y, Iwai A, Otero D, Kondo J, Ihara Y, Saitoh T: Molecular cloning of a novel component of amyloid in Alzheimer's disease. Proc Natl Acad Sci USA 1993, 90:11282–11286
2. Iwai A, Yoshimoto M, Masliah E, Saitoh T: Non-Aβ component of Alzheimer's disease amyloid (NAC) is amyloidogenic. Biochemistry 1995, 34:10139–10145
3. Masliah E, Iwai A, Mallory M, Ueda K, Saitoh T: Altered presynaptic protein NACP is associated with plaque formation and neurodegeneration in Alzheimer's disease. Am J Pathol 1996, 148:201–210